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(54) Title: BROADBAND ABSORBING FILM FOR LASER CAPTURE MICRODISSECTION

(57) Abstract

A thermoplastic film for LCM tissue transfer is thermally coupled to a broadband energy-absorbing material. The broadband energy-absorbing material may either be introduced into the film composition as a dopant or may be in thermal contact with the film.



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## SPECIFICATION

BROADBAND ABSORBING FILM FOR  
LASER CAPTURE MICRODISSECTION

## BACKGROUND OF THE INVENTION

## 1. Field of the Invention

The present invention relates to laser capture microdissection. More particularly, the present invention relates to broadband absorbing films for use in laser capture microdissection.

## 2. The Prior Art

The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp. 998-1001, published in 1996, incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electrocoat Corporation of Pompton Lakes, New Jersey

(type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90 C.

5 The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film  
10 manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

15 While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

20 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

25 It is therefore an object of the invention to provide a film for LCM applications which overcomes the shortcomings of the prior art.

It is another object of the present invention to provide a simple LCM film to be used in an LCM instrument.

30 Yet another object of the present invention is to provide an LCM film which is very thin and can have very high optical absorption.

A further object of the present invention is to provide an optical quality LCM film which is not sensitive to the wavelength of the incident laser light.

35 A further object of the present invention is to provide optical quality LCM films of adjustable thickness.

Another object of the present invention is to provide an LCM film whose optical density can be adjusted so that sufficient light is transmitted through the sample to permit the sample to be viewed with back light illumination.

5

Yet another object of the present invention is to provide an LCM film which does not use an organic dye that might have detrimental photochemistry effects.

10

Yet another object of the present invention is to provide an LCM film which does not employ an organic dye which does not have to be dissolved into the EVA polymer at high concentrations.

#### BRIEF DESCRIPTION OF THE INVENTION

15

A thermoplastic film for LCM tissue transfer is thermally coupled to a broadband energy-absorbing material. The broadband energy-absorbing material may either be introduced into the film composition as a dopant or may be in thermal contact with the film.

20

According to one embodiment of the present invention, a film for LCM tissue transfer comprises a layer of a transparent support film; a layer of a broadband absorbing film; and a layer of a low temperature thermoplastic; the absorbing film having an overall thickness chosen to absorb a desired fraction of incident laser light.

25

According to this embodiment of the invention, the film consists of a sandwich of layers containing a transparent support film such as mylar or polyester, a broadband absorbing film such as a metal film of nichrome or titanium, and a low temperature thermoplastic layer such as ethyl vinyl alcohol (EVA). The metal film thickness is chosen so as to absorb a desired fraction of incident laser light. A typical film may absorb say 10% to 90% of the incident light. The metal thickness is adjusted to provide this optical density by controlling the length of time of the sputtering deposition process. The metal absorbing layer can be sandwiched between two layers of EVA if desired.

30

35

A method for fabricating a multilayer film for LCM tissue transfer comprises the steps of: providing a support layer; forming a thin layer of a broadband

absorbing film onto the support layer, the broadband absorbing film having a thickness chosen to absorb a desired fraction of incident laser light; and coating the broadband absorbing film with a layer of thermoplastic.

5           According to the method of the present invention, one embodiment of the film is fabricated by evaporating a thin layer of metal film onto the polyester support using a deposition technique such as sputtering. The film thickness is chosen so as to absorb a desired fraction of incident laser light, usually between 10% and 95%. The metal thickness is adjusted to provide this optical density by controlling the  
10           length of time of the sputtering deposition process. The polyester/metal film is then coated with a thin layer (a few to 100 microns) of EVA thermoplastic using a spin coater to achieve a uniform layer across the surface. The spin coating process can be repeated several times to adjust the thickness of the EVA film.

15           According to one embodiment of the present invention, a thermoplastic film is doped with a broadband energy-absorbing material. The dopant concentration is adjusted so as to give a suitable absorption, usually between 10% and 95%, for the desired thickness of EVA film.

20           According to another embodiment of the present invention, a broadband absorbing material is deposited on a substrate such as a cap for a biological analysis vessel. The metal film is deposited onto the bottom of a cap and then the cap is coated with EVA using the process that is disclosed in the prior application.

25           BRIEF DESCRIPTION OF THE DRAWING FIGURES

FIG. 1 is a cross sectional view of an LCM tissue transfer film in a multilayer structure according to a first embodiment of the present invention.

30           FIG. 2 is a cross sectional view of an LCM tissue transfer film in a multilayer structure according to a second embodiment of the present invention.

FIG. 3 is a cross sectional view of an LCM tissue transfer film according to a second embodiment of the present invention wherein a broadband energy-absorbing material is doped into the transfer film.

35

FIG. 4 is a cross sectional view of an LCM tissue transfer film according to a second embodiment of the present invention wherein a broadband energy-absorbing material is formed on a substrate

5        FIG. 5 is a perspective view of a cap for an analysis vessel showing a procedure for affixing the LCM film thereto.

FIG. 6 is a perspective view of a cap for an analysis vessel showing an alternate procedure for affixing the LCM film thereto.

10

#### DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Those of ordinary skill in the art will realize that the following description of the present invention is illustrative only and not in any way limiting. Other embodiments of the invention will readily suggest themselves to such skilled persons.

15

Reference is made in the following disclosure to ethyl vinyl acetate (EVA) film as the transfer film. Persons of ordinary skill in the art will recognize that other films having desirable properties for use as the transfer film may also be employed for that purpose and the disclosure is not intended to be limited to LCM films employing EVA transfer films.

20

The present invention comprises an LCM film employing a broadband energy-absorbing material either doped into the layer of transfer film material or as a film in thermal contact with the layer of transfer film material. The broadband absorber should have a relatively broad absorption line throughout the visible region of the spectrum so that the absorber does not effect the color spectrum of the transmitted light that is used to illuminate the sample.

25

Metal films, such as nichrome or titanium are examples of a broadband absorber that can be deposited on a surface which can be thermally coupled to the transfer film. Thermal coupling to the transfer film may be accomplished by evaporating a thin layer of broadband absorbing film onto a transparent support film such as mylar or polyester.

30

35



Referring first to FIG. 1, a cross sectional view of an LCM tissue transfer film 10 according to a first embodiment of the present invention is presented. The film 10 consists of a sandwich of layers including a transparent support film 12 such as mylar or polyester, a broadband absorbing film 14 such as a metal film of nichrome or titanium, and a low temperature thermoplastic layer 16 such as EVA.

The transparent support layer may have a thickness of between about 25 microns and about 250 microns. The thickness of the broadband absorbing film is chosen so as to absorb a desired fraction of incident laser light. A typical film may absorb from about 10% to about 95% of the incident light. The metal thickness is adjusted to provide this optical density by controlling the length of time of the sputtering deposition process.

The film 10 of FIG. 1 is fabricated by evaporating a thin layer of broadband absorbing film onto the support film using a deposition technique such as sputtering as is known in the art. Another method for depositing metal film coatings is to use electron beam evaporation methods which are well known in the art. The appropriate thickness of the metal film can be determined by monitoring the film deposition process using a witness sample that allows measurement of the optical transmission of the film as a function of deposition time. The deposition is halted when the appropriate transmission level is reached. This is a procedure that is well known to those skilled in the art of thin film coating.

According to a presently preferred embodiment of the invention, the support and broadband absorbing film sandwich is then coated with a thin layer (between a few microns and about 100 microns) of EVA thermoplastic using a spin coater to achieve a uniform layer across the piece. The spin coating process can be repeated several times to adjust the thickness of the EVA transfer film. If necessary, the EVA material may be dissolved in a solvent such as methylene chloride to reduce its viscosity as is well known to those of ordinary skill in the art.

Referring now to FIG. 2, a cross sectional view of an LCM tissue transfer film 20 according to a second embodiment of the present invention is presented. The film 20 consists of a sandwich of layers including a transparent support film 22 such as mylar or polyester, a first low temperature thermoplastic layer 24 such as EVA, a

broadband absorbing film 26 such as a metal film of nichrome or titanium, and a second low temperature thermoplastic layer 28 such as EVA.

5 The process for making the film of FIG. 2 is similar to that used to make the film of FIG. 1. The additional EVA layer may be formed by a spin-on process.

10 In use, the EVA side of the film of either FIGS. 1 or 2 is placed in contact with a thin tissue sample. Laser light is focused on the sandwich structure and the light is absorbed by the thin metal film, which raises the temperature of the EVA film and melts the portion of the EVA that is in contact with the region of the metal film that is exposed to the focused laser beam. The laser power is chosen that the temperature rise does not melt the polyester support film. For films contemplated by the present invention, typical laser powers are on the order of 50mW for film transmissions of about 10%. The melted EVA area adheres to tissue samples which can then be excised by removing the film.

15 Referring now to FIG. 3, a cross sectional view is presented of an LCM tissue transfer film 30 according to a second embodiment of the present invention wherein a broadband energy-absorbing material is doped into the transfer film. The broadband energy-absorbing material is illustrated as shaded region 32.

20 An example of a broadband absorber that can be used to dope the EVA plastic is the chemical substance Buckminsterfullerene available as product #379646 from Sigma Chemical Company of St. Louis, Mo. This substance is a pure carbon compound comprising submicron particles that have very broad absorption profiles in the visible region of the spectrum. The Buckminsterfullerene can be mixed with heated EVA and the concentration adjusted so as to give a suitable absorption, usually between 10% and 95%, for the desired thickness of EVA film. The advantage of this method of doping the film is that, unlike the prior art LCM transfer films containing organic dyes, a specific laser wavelength is not required for activation and the extinction coefficient of the Buckminsterfullerene is very high. Moreover, photochemical effects should be reduced compared to those produced using an organic dye.

30 FIG. 4 is a cross sectional view of an embodiment of the present invention wherein a broadband energy-absorbing material 40 is formed on a substrate 42 and

a thermoplastic LCM transfer film 44 is affixed to the surface of the broadband energy-absorbing material. The broadband energy-absorbing material 40 is preferably a metal film such as nichrome or titanium. As may be seen from FIG. 4, substrate 42 is in the shape of a cap for a biological analysis vessel as disclosed in co-pending application serial No. \_\_\_\_\_, filed February 7, 1997, entitled "LASER CAPTURE MICRODISSECTION ANALYSIS VESSEL" expressly incorporated herein by reference. The biological analysis vessel may be a vessel such as an eppendorf tube or other well-known vessel.

FIG. 5 is a perspective view of a cap 42 for an analysis vessel illustrating a step in a first procedure for affixing the LCM film thereto. Persons of ordinary skill in the art will observe that cap 42 is equipped with a marking means such as a UPC label or laser etched label. Serializing all of the caps provides for easy identification and tracking of cell samples. The label may be read by a sensor, such as a UPC label sensor or OCR sensor which is mounted in or on the laser capture microdissection apparatus.

According to this aspect of the present invention, the serial number is placed on the top of the cap. The serial number is placed on the top of cap 42, and the thickness of cap 42 is selected to be larger than the depth of field of the microscope objective of the LCM apparatus with which cap 42 will be used. Thus, the microscope can be focussed on the tissue sample below the bottom surface of the cap and not have the label or serial number interfere optically, since the label is far from the focal plane of the imaging lens and is thus out of focus.

There are several ways in which the LCM transfer film 44 may be affixed to the surface of the caps according to the present invention. As may be seen from FIG. 5, first, a small, e.g., about 1 cm square piece of thermoplastic film 44 is cut. The film 44 is gently pressed onto the bottom surface of the cap 42 making it stick thereto. A glass microscope slide 46 is heated to about 100 C on a hot plate. A 0.002 inch thick piece of mylar plastic release liner 48 is placed on the slide. As is known in the art, a release liner is a plastic sheet that is coated with a silicone coating so it does not stick to the thermoplastic film material or the glass slide.

The cap 42 with its attached film 44 is pressed onto the release liner/slide assembly for about 5 seconds or until the film melts. The cap 42 with attached film

44 and release liner 48 is then removed from the hot glass slide 46, cooled down to room temperature, and the release liner 48 is peeled off. Finally, the excess thermoplastic film is trimmed off.

5           FIG. 6 is a perspective view of a cap for an analysis vessel showing an alternate procedure for affixing the LCM film thereto. According to this method, a piece of transparent double sided adhesive tape 50 (such as standard double stick tape available from 3M Corporation) may be used to tape the thermoplastic film 44 to the bottom of the cap 42. The excess thermoplastic film may then be trimmed off.

10           There are several advantages inherent in the LCM transfer film of the present invention. The metal film layer can be very thin and still have very high optical absorption. The spin coating process gives optical quality films of adjustable thickness. In addition, the film of the present invention is not sensitive to the  
15           wavelength of the incident laser light as are the prior art dye impregnated films.

          Another advantage of the present invention is that the broadband energy-absorbing material may be isolated from the tissue sample by the EVA transfer film layer. The broadband energy-absorbing material of the present invention does not  
20           use an organic dye that might have detrimental photochemistry effects on the sample with which it is in contact. Also, in embodiments of the present invention which employ a metal film, the metal film does not have to be dissolved into the EVA polymer at high concentrations like an organic dye in the prior art films.

25           While embodiments and applications of this invention have been shown and described, it would be apparent to those skilled in the art that many more modifications than mentioned above are possible without departing from the inventive concepts herein. The invention, therefore, is not to be restricted except in the spirit of the appended claims.

What is claimed is:

1. A LCM tissue transfer film comprising a thermoplastic film thermally coupled to a broadband energy-absorbing material.

5 2. The LCM transfer film of claim 1 wherein said broadband energy-absorbing material absorbs from about 10% to about 95% of incident light.

3. The LCM tissue transfer film of claim 1 wherein said broadband energy-absorbing material is introduced into the film composition as a dopant.

10 4. The LCM transfer film of claim 3 wherein said broadband energy-absorbing material comprises Buckminsterfullerine.

15 5. The LCM transfer film of claim 3 wherein said broadband energy-absorbing material absorbs from about 10% to about 95% of incident light.

20 6. The LCM tissue transfer film of claim 1 wherein said thermoplastic film is an ethyl vinyl acetate (EVA) film and said broadband energy-absorbing material is introduced into the film composition as a dopant.

7. The LCM tissue transfer film of claim 6 wherein said dopant comprises Buckminsterfullerine.

25 8. The LCM tissue transfer film of claim 6 wherein said thermoplastic film has a thickness of about 100 microns.

9. The LCM tissue transfer film of claim 1 wherein said broadband energy-absorbing material is a layer in thermal contact with the film.

30 10. The LCM tissue transfer film of claim 9 wherein said broadband energy-absorbing material is a metal film layer.

35 11. The LCM tissue transfer film of claim 9 wherein said broadband energy-absorbing material is a titanium film layer.

12. The LCM transfer film of claim 11 wherein said broadband energy-absorbing material absorbs from about 10% to about 95% of incident light.

5 13. The LCM tissue transfer film of claim 10 wherein said broadband energy-absorbing material is a nichrome film layer.

14. The LCM transfer film of claim 13 wherein said broadband energy-absorbing material absorbs from about 10% to about 95% of incident light.

10 15. The LCM tissue transfer film of claim 1 wherein said thermoplastic film is an ethyl vinyl acetate (EVA) film and said broadband energy-absorbing material is a layer in thermal contact with the film.

15 16. The LCM tissue transfer film of claim 15 wherein said thermoplastic film has a thickness of about 100 microns.

17. The LCM tissue transfer film of claim 15 wherein said broadband energy-absorbing material is a metal film layer.

20 18. The LCM tissue transfer film of claim 15 wherein said broadband energy-absorbing material is a titanium film layer.

25 19. The LCM transfer film of claim 18 wherein said broadband energy-absorbing material absorbs from about 10% to about 95% of incident light.

20. The LCM tissue transfer film of claim 17 wherein said broadband energy-absorbing material is a nichrome film layer.

30 21. The LCM transfer film of claim 20 wherein said broadband energy-absorbing material absorbs from about 10% to about 95% of incident light.

35 22. A film for LCM tissue transfer comprising:  
a layer of a transparent support film;  
a layer of a broadband absorbing film; and  
a layer of a low temperature thermoplastic;

said film having an overall thickness chosen to absorb a desired fraction of incident laser light.

23. The film of claim 22 wherein:

5       said layer of a transparent support film comprises a film chosen from the group including mylar and polyester;

      said layer of a broadband absorbing film; comprises a metal film chosen from the group including nichrome and titanium, and

10       said layer of a low temperature thermoplastic comprises ethyl vinyl alcohol (EVA).

24. The film of claim 22 wherein said desired fraction of incident laser light is between about 10% to about 90%.

15       25. A film for LCM tissue transfer comprising:

      a layer of a transparent support film;

      a first of a low temperature thermoplastic;

      a layer of a broadband absorbing film; and

      a second layer of a low temperature thermoplastic;

20       said absorbing film having an overall thickness chosen to absorb a desired fraction of incident laser light.

26. The film of claim 25 wherein:

25       said layer of a transparent support film comprises a film chosen from the group including mylar and polyester;

      said layer of a broadband absorbing film; comprises a metal film chosen from the group including nichrome and titanium, and

      said first and second layers of a low temperature thermoplastic comprise ethyl vinyl alcohol (EVA).

30

27. The film of claim 25 wherein said desired fraction of incident laser light is between about 10% to about 90%.

28. A carrier for LCM analysis comprising:

35       a substrate; and

an a LCM film mounted on said substrate, said LCM film comprising a thermoplastic film thermally coupled to a broadband energy-absorbing material..

29. The carrier of claim 28 wherein said carrier is transparent at least in a region where said film is mounted thereon.

30. The carrier of claim 28 wherein said carrier comprises a plastic material.

31. The carrier of claim 28 wherein said substrate is adapted to mate with an analysis vessel.

32. The carrier of claim 28 wherein said substrate is adapted to mate with a holder in an LCM tissue sample transfer apparatus.

33. A cap for sealing a laser capture microdissection sample vessel having an open end, said cap comprising:

a plastic member adapted to mate with the open end of said sample vessel so as to seal said vessel, said member having a top surface and a bottom surface and further having an aperture formed from said top surface to said bottom surface; and

a laser capture microdissection lift-off substrate mounted on said bottom surface across and sealing said aperture.

34. The cap of claim 33 having a thickness between said top surface and said bottom surface selected to be larger than the depth of field of a selected microscope objective, said cap further having a label on said top surface.

35. A cap for sealing a laser capture microdissection sample vessel having an open end, said cap comprising:

a plastic member adapted to mate with the open end of said sample vessel so as to seal said vessel, said member having transparent optical quality windows on opposing top and bottom surfaces defining a solid volume thereof; and

a laser capture microdissection lift-off substrate affixed to said window on said bottom surface.



36. The cap of claim 13 having a thickness between said top surface and said bottom surface selected to be larger than the depth of field of a selected microscope objective, said cap further having a label on said top surface.

5           37. A method for fabricating a film for LCM tissue transfer comprising the steps of:

          providing a support layer;

          forming a layer of a broadband absorbing film onto said support layer, said broadband absorbing film having a thickness chosen to absorb a desired  
10       fraction of incident laser light;

          coating said layer of broadband absorbing film with a layer of thermoplastic.

          38. A method for fabricating a film for LCM tissue transfer comprising the  
15       steps of:

          providing a polyester support layer:

          forming a thin layer of metal film onto said polyester support layer, said metal film having a thickness chosen to absorb a desired fraction of incident laser  
light;

20           coating said metal film with a layer of EVA thermoplastic.

          39. The method of claim 38 wherein said forming step comprises a sputtering step and said coating step comprises at least one spin coating step.

25           40. The method of claim 39 wherein said coating step comprises a plurality of spin coating steps.

          41. A method for fabricating a film for LCM tissue transfer comprising the steps of:

30           providing a support layer;

          forming a first layer of thermoplastic on said support layer;

          forming a layer of a broadband absorbing film onto said first layer of thermoplastic, said broadband absorbing film having a thickness chosen to absorb a desired fraction of incident laser light;

35           coating said layer of broadband absorbing film with a second layer of thermoplastic.

42. A method for fabricating a film for LCM tissue transfer comprising the steps of:

providing a polyester support layer:

5 forming a first layer of EVA thermoplastic on said support layer;

forming a thin layer of metal film onto said first layer of EVA thermoplastic, said metal film having a thickness chosen to absorb a desired fraction of incident laser light;

10 coating said metal film with a second layer of EVA thermoplastic.

43. The method of claim 42 wherein said forming step comprises a sputtering step and said coating step comprises at least one spin coating step.

15 44. The method of claim 42 wherein said coating step comprises a plurality of spin coating steps.

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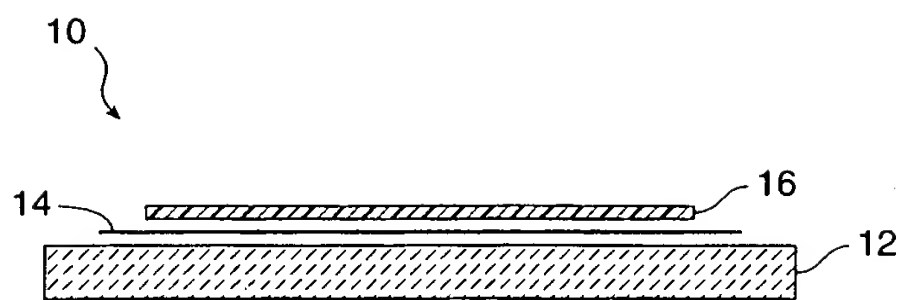


FIG. 1

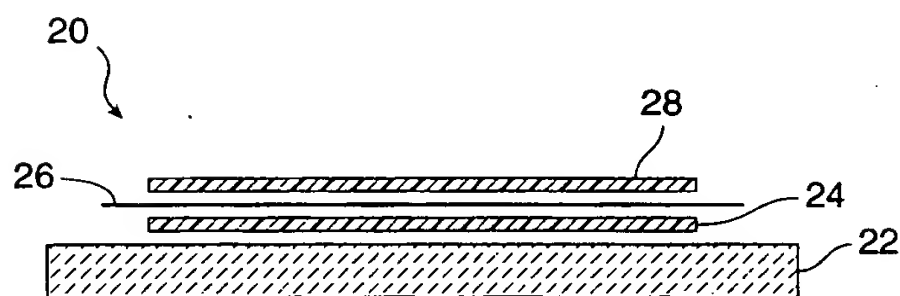


FIG. 2

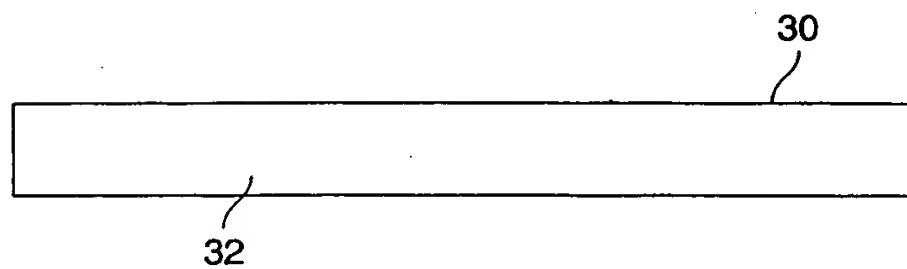


FIG. 3

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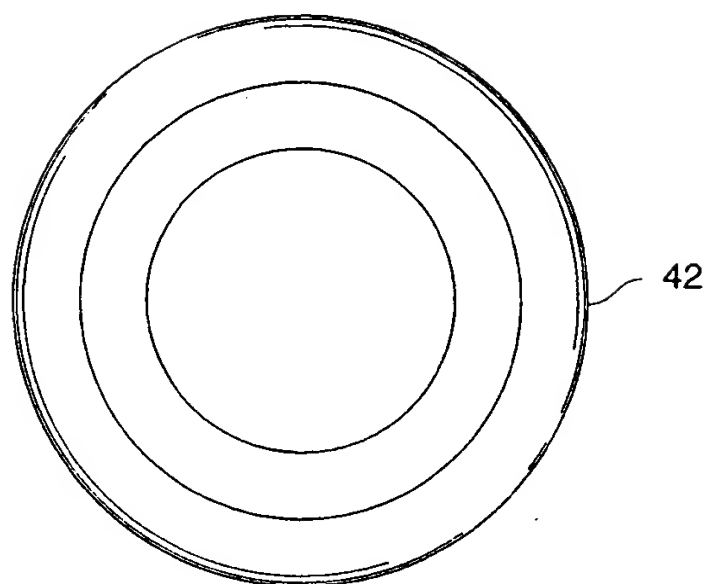


FIG. 4A

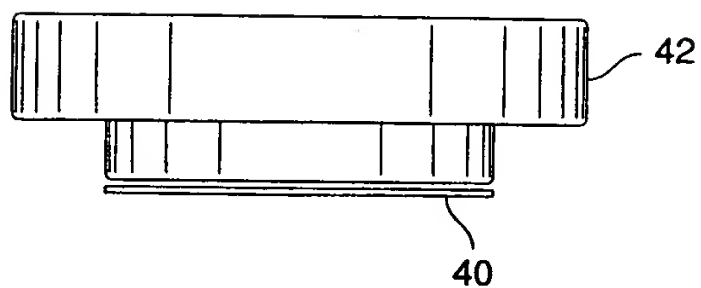


FIG. 4B

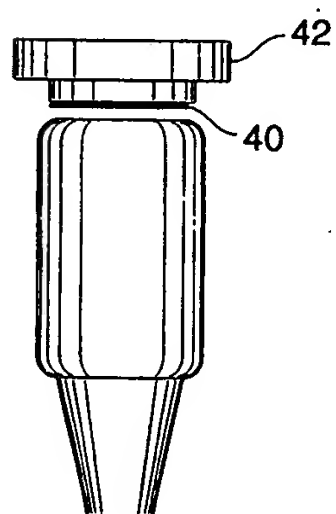
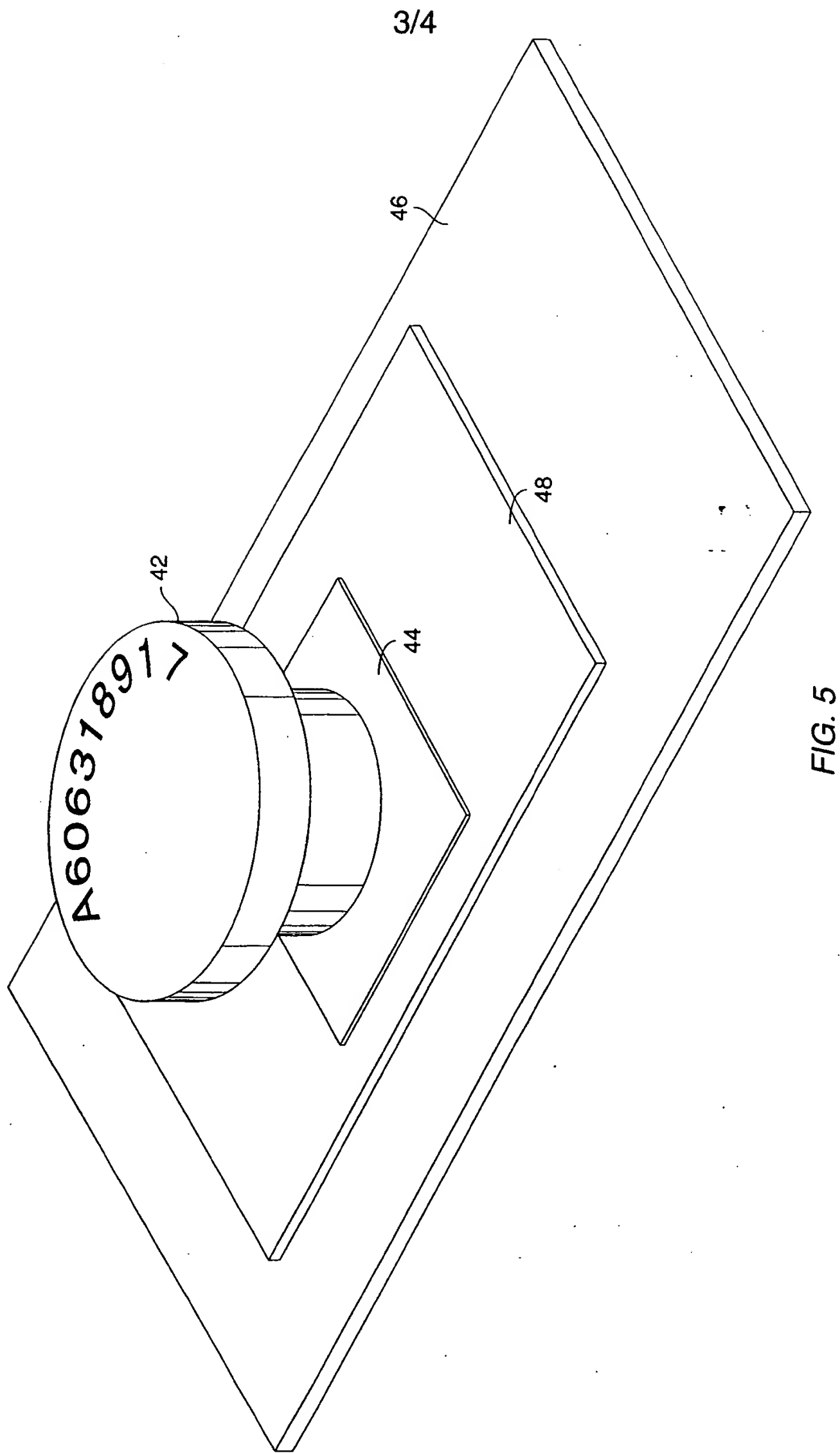


FIG. 4C



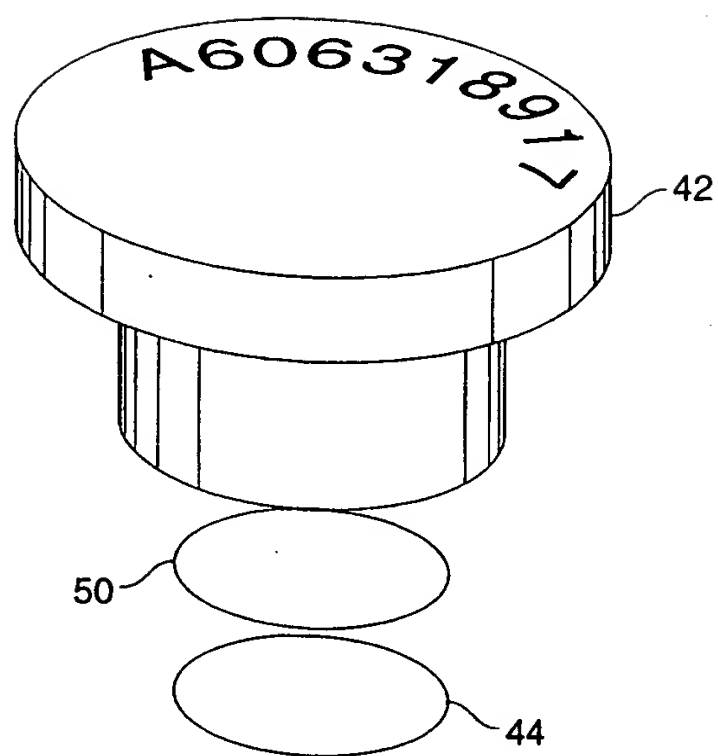


FIG. 6

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01634

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N1/28 B01L3/14

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N B01L G02B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EMMERT-BUCK M R ET AL: "LASER CAPTURE MICRODISSECTION" SCIENCE, vol. 274, no. 5289, 8 November 1996, pages 998-1001, XP000644727 cited in the application see the whole document	1,4,6,7, 28,33-36
A	---	22,25, 37,38, 41,42
Y	US 5 574 077 A (DOUGHERTY THOMAS K ET AL) 12 November 1996 see abstract	1,4,6,7, 28
Y	EP 0 081 976 A (STERILIN LTD) 22 June 1983 see abstract	33,34
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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- "&" document member of the same patent family

Date of the actual completion of the international search

11 June 1998

Date of mailing of the international search report

22/06/1998

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01634

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International Application No

PCT/US 98/01634

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